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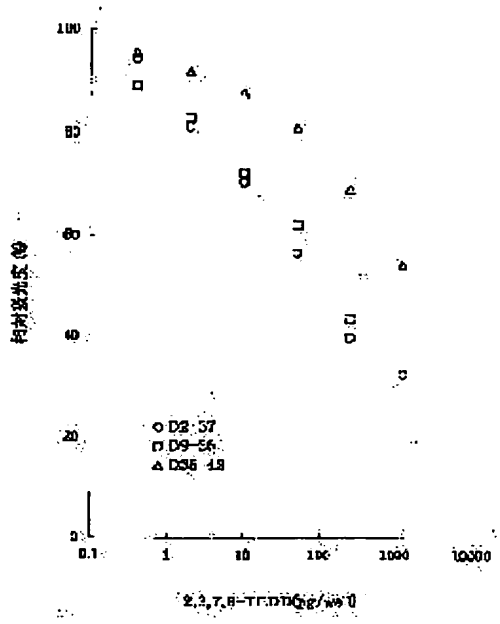
(54) MONOCLONAL ANTIBODY AGAINST DIOXIN AND ITS USE



(図4) 2,3,7,8-TCDD の標準曲線

(57)Abstract:

PROBLEM TO BE SOLVED: To establish a simple and high-sensitivity enzyme immunoassay method for dioxins in a human biological sample.

SOLUTION: Dioxins in a human biological sample are detected and assayed by an enzyme immunoassay method by a monoclonal antibody which is specific to one or more dioxins and has the maximum affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin among dioxins.

LEGAL STATUS

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CLAIMS

[Claim(s)]
[Claim 1] The monoclonal antibody whose compatibility it is a more specific monoclonal antibody than 1 or it of dioxin, and is max among dioxin at 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin.
[Claim 2] The monoclonal antibody according to claim 1 whose dioxin is Polyhalogenation dibenzo-p-dioxin or a Polyhalogenation dibenzofuran.
[Claim 3] The monoclonal antibody according to claim 1 or 2 whose dioxin is the Polyhalogenation-p-dioxin of following the (1) - (7), or the Polyhalogenation dibenzofuran of following the (8) - (17).
(1) 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin (2378TCDD), (2) 1, 2, 3, 7, 8-pentachloro dibenzo-p-dioxin (12378PeCDD), (3) 1, 2, 3, 4, 7, 8-hexachloro dibenzo-p-dioxin (123478HxCDD), (4) 1, 2, 3, 6, 7, 8-hexachloro dibenzo-p-dioxin (123678HxCDD), (5) 1, 2, 3, 7, 8, 9-hexachloro dibenzo-p-dioxin (123789HxCDD), (6) 1, 2, 3, 4, 6, 7, 8-heptachloro dibenzo-p-dioxin (1234678HpCDD), (7) 1, 2, 3, 4, 6, 7, 8, 9-octachloro dibenzo-p-dioxin (12346789OCDD), (8) 2, 3, 7, 8-tetrachlorodibenzofuran (2378TCDF), (9) 1, 2, 3, 7, 8-pentachloro dibenzofuran (12378PeCDF), (10) 2, 3, 4, 7, 8-pentachloro dibenzofuran (23478PeCDF), (11) 1, 2, 3, 4, 7, 8-hexachloro dibenzofuran (123478HxCDF), (12) 1, 2, 3, 6, 7, 8-hexachloro dibenzofuran (123678HxCDF), (13) 1, 2, 3, 7, 8, 9-hexachloro dibenzofuran (123789HxCDF), (14) 2, 3, 4, 6, 7, 8-hexachloro dibenzofuran (234678HxCDF), (15) 1, 2, 3, 4, 6, 7, 8-heptachloro dibenzofuran (1234678HpCDF), (16) 1, 2, 3, 4, 7, 8, 9-heptachloro dibenzofuran (1234789HpCDF), (17) 1, 2, 3, 4, 6, 7, 8, 9-octachloro dibenzofuran (12346789OCDF).
[Claim 4] Furthermore, the monoclonal antibody according to claim 3 whose compatibility of (2), (8), or (10) is size.
[Claim 5] The monoclonal antibody according to claim 4 whose rate of a crossover of (2) and (10) is before and after 0.5 when compatibility of (1) is set to 1.0.
[Claim 6] The monoclonal antibody according to claim 4 0.2 order and whose rate of a

crossover of (8) and (10) are before and after 0.3 for the rate of a crossover of (2) when compatibility of (1) is set to 1.0.

[Claim 7] The monoclonal antibody according to claim 4 whose rate of a crossover of (2) is before and after 1.0 when compatibility of (1) is set to 1.0.

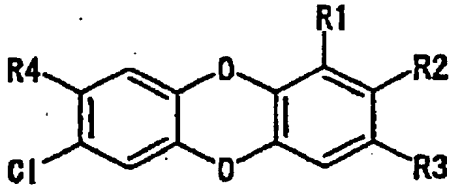
[Claim 8] The monoclonal antibody according to claim 5 which is what is produced from a hybridoma D 9-36 (FERM P-18057).

[Claim 9] The monoclonal antibody according to claim 6 which is what is produced from a hybridoma D 2-37 (FERM P-18056).

[Claim 10] The monoclonal antibody according to claim 7 which is what is produced from a hybridoma D 35-42 (FERM P-18058).

[Claim 11] Carry out immunity of the animal with the connective of the hapten and the protein which are expressed with the following formulas, and an antibody manufacture cell is obtained from this animal. Unite said cell with a tumor cell and two or more hybridomas (mixed kind) are made to generate. The manufacture approach of a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, or 7 which chooses at least one sort of hybridomas which manufacture dioxin and the antibody which reacts from these two or more hybridomas, and is characterized by collecting the antibodies manufactured from this hybridoma.

[Formula 1]



R1 among a formula NHCO(CH2)2COOH, NHCO(CH2)3COOH, NHCO(CH2)4COOH, NHCOCH=CHCOOH, CH=CHCOOH, It is the functional group chosen from CH=C(CH3) COOH, 2(CH=CH) COOH, and H. R2 is a functional group chosen from H, Cl, OCH2COOH, O(CH2)3COOH, O(CH2)4COOH, CH=CHCOOH, and 2(CH=CH) COOH, R3 is Cl or H, and R4 is Cl, CH3, or C (CH3)3.

[Claim 12] The manufacture approach of a monoclonal antibody according to claim 11 that the proteins in the hapten-protein connective used by claim 11 are bovine serum albumin, ovalbumin, and a SUKASHI shellfish hemocyanin.

[Claim 13] The manufacture approach of a monoclonal antibody according to claim 11 that a hybridoma is D 9-36 (FERM P-18057), D 2-37 (FERM P-18056), or D 35-42 (FERM P-18058).

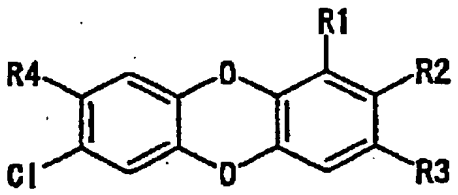
[Claim 14] The hybridoma which is obtained in claim 11 and which produces a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, or 7.

[Claim 15] The hybridoma according to claim 14 which is D 9-36 (FERM P-18057), D 2-37 (FERM P-18056), or D 35-42 (FERM P-18058).

[Claim 16] Detection of the dioxin by the immunity enzyme measuring method using a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, a measuring method.

[Claim 17] Enzyme-labeling hapten which combined the hapten expressed with the following formulas, and an enzyme and which is used for the assay system of dioxin.

[Formula 2]



R1 among a formula NHCO(CH2)2COOH, NHCO(CH2)3COOH, NHCO(CH2)4COOH, NHCOCH=CHCOOH, CH=CHCOOH, It is the functional group chosen from CH=C(CH3) COOH, 2(CH=CH) COOH, and H. R2 is a functional group chosen from H, Cl, OCH2COOH, O(CH2)3COOH, O(CH2)4COOH, CH=CHCOOH, and 2(CH=CH) COOH, R3 is Cl or H, and R4 is Cl, CH3, or C (CH3)3.

[Claim 18] Enzyme-labeling hapten according to claim 17 whose enzymes are horseradish peroxidase and the beta-galactosidase.

[Claim 19] 1) and (1) -- the solid phase which fixed the antibody to a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 -- (2) A monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, Enzyme-labeling hapten according to claim 17 is made to react. (3) -- the sample containing dioxin, and (4) -- 2) Detection of the dioxin according to claim 16 characterized by measuring the concentration of the dioxin in a sample by measuring the marker enzyme activity fixed by solid phase, measuring method.

[Claim 20] 1) and (1) -- the solid phase which fixed the monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 -- Enzyme-labeling hapten according to claim 17 is made to react. (2) -- the sample containing dioxin, and (3) -- 2) Detection of the dioxin according to claim 16 characterized by measuring the concentration of the dioxin in a sample by measuring the marker enzyme activity fixed by solid phase, measuring method.

[Claim 21] The constituent which is a constituent used for high grade-ization of dioxin and comes to contain a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[Claim 22] Biochemical, immunological, functional, or the constituent that is a constituent used with other research analysis methods, and comes to contain the monoclonal antibody of an effective dose according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of

dioxin.

[Claim 23] The kit which is existence of the dioxin in a sample, or a kit for measurement of concentration, and contains a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 and enzyme-labeling hapten according to claim 17.

[Claim 24] Existence of the dioxin in the sample containing the antibody to a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, a monoclonal antibody according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, and enzyme-labeling hapten according to claim 17, or the kit for measurement of concentration.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

This invention relates to 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin among the monoclonal antibody to dioxin, especially dioxin at the monoclonal antibody whose compatibility is max. This invention relates to the manufacturing method of this monoclonal antibody, detection of the dioxin which uses this monoclonal antibody, a measuring method, and the kit for it again.

[0002]

[Description of the Prior Art]

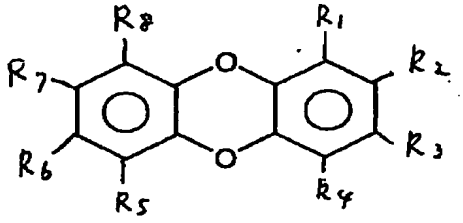
In recent years, due to concern of effect for the environmental pollution by the chemical, and those Homo sapiens health, about the dioxin contained in atmospheric air, incinerated ash, exhaust gas, wastewater, common water, food, etc., also not only in Japan but in every country in the world, it is social concerns, and the lasting toxic threat which the dioxin as an environmental pollutant does to a living thing and human beings is said. Although dioxin is the un-meaning-chemicals generated by the processing process accompanied by the heat of trash, the process of production of an organochlorine compound, etc., since a generation source is various and the environmental pollution has posed a big problem, it is pressing need to investigate exposure in Homo sapiens, and the ecosystem and the contamination situation of food by these. The developmental toxicity of dioxin has the big problem of the effect being irreversible in many cases, and moreover attaining to the next generation the top where susceptibility is high, compared with a living body's toxicity. Since the effect on the child by the mother under pregnancy and breast-feeding being exposed especially may appear also by very low-concentration dioxin, it is anxious for establishment of the microestimation which can act as the

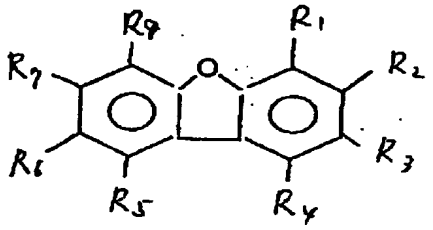
monitor of the amount of exposures exactly and quickly. In order to measure specific dioxin with high sensitivity and high degree of accuracy about the sample in which the pollutant of varieties is intermingled, a high-resolution gas chromatography / mass spectrometry (HRGS/HRMS) has been used conventionally. However, in order to measure by HRGS/HRMS, since the proficient researcher needs to work [long duration] in the laboratory which other phases took complicated clean-up actuation to, and was improved well using expensive equipment in every sample, the costs are expensive remarkable things. From now on, development of a cheap, simple, and high sensitivity measuring method will be strongly desired from it being predicted that the measuring object sample kind and number of dioxin increase increasingly. Immunoassay is in one of the approaches of replacing with this HRGS/HRMS. Although some measurement of the dioxin by this approach is reported the sample (an environmental sample and standard solution) containing high-concentration dioxin -- it is (Kennel, S.J. et al. --) Toxicol.Appl.Pharmacol.1986, 82,256-263; Stanker, L.H. et al., Toxicology.1987, 45,229-243; Vanderlaan, M. et al., Toxicol.Chem.1988, 7,859-870; Sugawara, Y. et al., Anal.Chem.1998, 70, 1092-1099; Sanborn, J.R. et al., J. Agric.FoodChem.1988, 46, 2407-2416; Harrison, R.O. et al., Organohalogen Compounds 1999, 36,129-132; Zennegg, M. et al., About a biological material with low dioxin contents, such as Homo sapiens mother's milk, blood, etc. with which Organohalogen Compounds 1999, 36,317-319, and measurement are called for, there is almost no report.

[0003]

Although dioxin will point out the polychlorinated dibenzofuran (PCDFs) shown in the dioxin [Polychlorination dibenzo-p-dioxin (PCDDs)] shown in the following formulas (a), and a formula (b) if dioxin is explained further These are tricyclic chlorination compounds, and many structural isomers exist by the number of permutation chlorine, or the difference in a location, and respectively, a biological operation and a physicochemical quality have a similar thing and a completely different thing, and also change toxicity remarkably with each isomer. Thus, since toxicity differs greatly between an isomer or a homolog, it is difficult to evaluate toxicity by observation concentration.

[Formula 3]





$R_1 \sim R_8$：HまたはCl
但し全てがHである場合を除く。

[0004]
Although there are 75 sorts of Polychlorination dibenzo-p-dioxin (PCDDs) and 135 sorts of polychlorinated dibenzofurans (PCDFs) in the structural isomer of dioxin The relative toxicity when setting the toxicity over 2, 3, 7 with the strongest toxicity, and 8-TCDD to 1, since the toxicity between each isomer is greatly different, A toxic equivalence multiplier (Toxic Equivalency Factor;TEF) is advocated and let seven sorts of strong toxic PCDDs(es), and ten sorts of PCDFs(es) be the measuring objects. Moreover, four sorts of non alt.PCBs and eight sorts of mono-alt.PCBs have come to be measured as a compound in which the same living thing operation as dioxin is shown among the polychlorinated biphenyl (PCBs) known as a contaminant in an environment for some time. So, total of the value which multiplied the observation concentration of each isomer by TEF is converted into a 2, 3, 7, 8-TCDD toxicity equivalent (Toxic Equivalent quantity;TEQ), and toxic evaluation is carried out to evaluation of dioxin using this value.
[0005]
Very recently it becomes clear for the great portion of TEQ of the dioxin in mother's milk to be occupied by three sorts of (1) 2, 3, 7, 8-TCDD, (2) 1, 2, 3, 7, 8-PeCDD and (10) 2, 3, 4, 7, 8-PeCDF. it was shown that the toxicity of dioxin can be evaluated by enzyme immunoassay (ELISA) which uses an antibody with high compatibility for these (Nakazawa, H. et al. --) Organohalogen Compounds 2000, 45, 86-89; Saito, K. et al., Organohalogen Compounds2000, 45,168-171; Sugawara, Y. et al., Organohalogen Compounds 2000, 45,172-175. However, since the antibody used here was a polyclonal

antibody of a rabbit, it is impossible for supplying an antibody without the lot difference at the time of production or acquisition in large quantities, and the problem was in the stability of the measuring method and a measurement result, and universality. Moreover, since two or more antibody kinds (clonotype) which participate in a reaction are intermingled even if it uses the antiserum of the same lot, the antibody kinds which react depending on a test portion may differ, and measured value may be changed. The measuring method of dioxin using the monoclonal antibody and it to dioxin is also proposed that the fault of the approach using the above-mentioned polyclonal antibody should be improved [refer to JP,63-14691,A (USP No. 4,798,807) and JP,63-74494,A]. These monoclonal antibodies have the indefinite information about the singularity and anti-dioxin antibody titer to dioxin. However, as a measuring method A thing given in JP,63-74494,A Since cross-reactivity with other dioxin homologs is not shown, from a toxic viewpoint, cannot evaluate the measurement result searched for using this antibody, and The toxicity of the thing of JP,63-14691,A is the strongest. From 2, 3, 7, 8-TCDD, to other dioxin homologs Since compatibility is high, There is a fault of not correlating with a toxic equivalent the measured value of the real sample from which the mixing ratio of a homolog differs, and also It was the thing which cannot be used for the quantum of the real sample in which the limit of determination of 2, 3, 7, 8-TCDD is before and after 1ng, and both contain the dioxin of minute amounts, such as a biological material, and which there is a problem by ** and lacks in practicality.

[0006]

[Problem(s) to be Solved by the Invention]

The enzyme immunoassay method [high sensitivity simply / this invention / the dioxin in a human biological material / and] (ELISA) is established, and let it be a technical problem to offer the monoclonal antibody which was suitable for the purpose of offering the approach of **(ing) to the monitoring and situation investigation of contamination especially by the dioxin in Homo sapiens.

[0007]

[Means for Solving the Problem]

Nakazawa and others investigated about the functionality of each measurement result of the homolog of the dioxin in the Homo sapiens mother's milk which might be measured by HRGC/MS, and the toxic equivalent (TEQ) computed based on the toxic equivalence multiplier (TEF) in order to check about selection and felicity of the measuring object by the ELISA method. Consequently, especially two sorts, (2) 1, 2, 3, 7, 8-PeCDD, and (10) 2, 3, 4, 7, 8-PeCDF, especially were excellent in correlation with TEQ, and when three sorts including 2, 3, 7 with high TEF, and 8-TCDD could be measured by ELISA, it was checked that dioxin exposure evaluation by the Homo sapiens as a toxic equivalent reduced property can be performed. As a result of repeating research in order to obtain a specific

monoclonal antibody to the three above-mentioned sorts, this invention persons succeed in obtaining the new monoclonal antibody which has the maximum compatibility in 8-TCDD among [2, 3, and 7] dioxin, and reach this invention.

[0008]

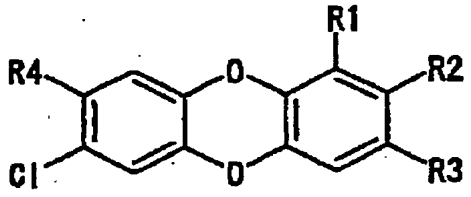
That is, this invention is a more specific monoclonal antibody than 1 or it of dioxin, and relates to 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin among dioxin at the monoclonal antibody whose compatibility is max. In this invention, dioxin points out Polyhalogenation dibenzo-p-dioxin or a Polyhalogenation dibenzofuran, and the Polyhalogenation-p-dioxin of following the (1) - (7) or the Polyhalogenation dibenzofuran of following the (8) - (17) is raised as these examples.

(1) 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin (2378TCDD), (2) 1, 2, 3, 7, 8-pentachloro dibenzo-p-dioxin (12378PeCDD), (3) 1, 2, 3, 4, 7, 8-hexachloro dibenzo-p-dioxin (123478HxCDD), (4) 1, 2, 3, 6, 7, 8-hexachloro dibenzo-p-dioxin (123678HxCDD), (5) 1, 2, 3, 7, 8, 9-hexachloro dibenzo-p-dioxin (123789HxCDD), (6) 1, 2, 3, 4, 6, 7, 8-heptachloro dibenzo-p-dioxin (1234678HpCDD), (7) 1, 2, 3, 4, 6, 7, 8, 9-octachloro dibenzo-p-dioxin (12346789OCDD), (8) 2, 3, 7, 8-tetrachlorodibenzofuran (2378TCDF), (9) 1, 2, 3, 7, 8-pentachloro dibenzofuran (12378PeCDF), (10) 2, 3, 4, 7, 8-pentachloro dibenzofuran (23478PeCDF), (11) 1, 2, 3, 4, 7, 8-hexachloro dibenzofuran (123478HxCDF), (12) 1, 2, 3, 6, 7, 8-hexachloro dibenzofuran (123678HxCDF), (13) 1, 2, 3, 7, 8, 9-hexachloro dibenzofuran (123789HxCDF), (14) 2, 3, 4, 6, 7, 8-hexachloro dibenzofuran (234678HxCDF), (15) 1, 2, 3, 4, 6, 7, 8-heptachloro dibenzofuran (1234678HpCDF), (16) 1, 2, 3, 4, 7, 8, 9-heptachloro dibenzofuran (1234789HpCDF), (17) 1, 2, 3, 4, 6, 7, 8, 9-octachloro dibenzofuran (12346789OCDF). This invention offers the monoclonal antibody which has the maximum (1) 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin, and has a compatibility with (2) 1, 2, 3, 7, 8-pentachloro dibenzo-p-dioxin, (8) 2, 3, 7, 8-tetrachloro dibenzofuran and/or (10) 2, 3, 4, 7, 8-pentachloro dibenzofuran. This invention offers the monoclonal antibody produced again from a hybridoma D 9-36 (FERM P-18057), a hybridoma D 2-37 (FERM P-18056), or a hybridoma D 35-42 (FERM P-18058).

[0009]

This invention carries out immunity of the animal again with the connective of the hapten and the protein which are expressed with the following formulas. Obtain an antibody manufacture cell from this animal, unite said cell with a tumor cell, and two or more hybridomas are made to generate. At least one sort of hybridomas which manufacture dioxin and the antibody which reacts are chosen from these two or more hybridomas, and it is related with the manufacture approach of a monoclonal antibody characterized by collecting the antibodies manufactured from this hybridoma.

[Formula 4]



R1 among a formula NHCO(CH2)2COOH, NHCO(CH2)3COOH, NHCO(CH2)4COOH, NHCOCH=CHCOOH, CH=CHCOOH, It is the functional group chosen from CH=C(CH3) COOH, 2(CH=CH) COOH, and H. R2 is a functional group chosen from H, Cl, OCH2COOH, O(CH2)3COOH, O(CH2)4COOH, CH=CHCOOH, and 2(CH=CH) COOH, R3 is Cl or H, and R4 is Cl, CH3, or C (CH3)3. As protein combined with the above-mentioned hapten, bovine serum albumin, ovalbumin, a SUKASHI shellfish hemocyanin, etc. are mentioned. Moreover, this invention relates to the hybridoma expressed especially with D 9-36 (FERM P-18057), D 2-37 (FERM P-18056), or D 35-42 (FERM P-18058) about the hybridoma which produces the above-mentioned monoclonal antibody. Furthermore, this invention is biochemical, immunological, functional, or the thing about the constituent used with other research analysis methods containing the monoclonal antibody of detection of the dioxin by the immunity enzyme measuring method which uses the monoclonal antibody of this invention, a measuring method, the high grade-ized constituent of the dioxin containing the monoclonal antibody of this invention, and this invention of dioxin. Moreover, it is related with existence of the monoclonal antibody of this invention and the dioxin in the sample containing enzyme-labeling hapten, or the kit for measurement of concentration. The monoclonal antibodies of this invention can be used for identification of the dioxin contained in the sample, and it can be used for the density measurement of the dioxin in a sample. As an object sample, biological materials, such as environmental samples, such as soil, water, atmospheric air, and fly ash, and blood, and mother's milk, etc. can be mentioned.

[0010]

When it uses as a reagent in the various immunity analysis methods for measuring existence of dioxin or concentration, an analysis method improves by use of the monoclonal antibodies of this invention. Singularity becomes high while becoming more conveniently and quickly [detection], and sharp. As an immunity analysis method which can use the monoclonal antibodies of this invention, although radioimmunoassay (RIA) (radioimmunity analysis), contention immunity precipitate analysis, enzyme connection immune absorbance analysis (ELISA), and immunofluorescence analysis can be mentioned, it is not limited only to these immunity analysis methods. Especially in this invention, using for an immunity enzyme measuring method is desirable, in that case, a false antigen is fixed and the indirect competing method make a reaction with the dioxin

in a sample and a monoclonal antibody compete, and the direct competing method for fixing an antibody and seeing a reaction rate with the dioxin in a sample and enzyme-labeling hapten are mentioned. Although the monoclonal antibody of this invention can also be used as an antibody in the latter, the second antibody to Mouse IgG is fixed, and the methods of making a sample, a monoclonal antibody, and enzyme-labeling hapten contact are points, such as the repeatability, simple nature, and sensibility, and it excels, and is desirable. Horseradish peroxidase, the beta-galactosidase, etc. are mentioned as an enzyme for carrying out the indicator of the hapten.

[0011]

The constituent by this invention which authorizes thru/or measures the existence of dioxin or concentration in a sample contains the antibody of effective concentration, in order to carry out the antibody of concentration effective in order to detect existence of a chemical, or the quantum of the amount of chemicals. Mixing or an antibody may be made for the suitable support and the suitable antibody like a latex particle or a plastics microtiter plate to adhere to such support. According to the immunologic procedure to be used, an enzyme or coloring matter can also be blended with an antibody, and radiolabeling can also be attached. Therefore, all the analysis methods using the dioxin containing 2, 3, 7, 8-TCDD and the monoclonal antibodies which react are included by the technical range of this invention. Based on an alternative immunoreaction, from complicated mixture or a solution, the monoclonal antibodies of this invention can use dioxin measurement, isolation, purification, and/or in order to remove. By use of dioxin and the monoclonal antibody which reacts, a conventional method is improved remarkably. The properties that use these monoclonal antibodies for the above-mentioned reaction, and usefulness is demonstrated are the remarkable singularity accepted in comparison with polyclonal antibodies, and the large-scale industrial thing which it is, and it carries out, and use of the homogeneous antibody in a commercial scale is enabled, and can come to hand without a limit quantitatively in practice.

[0012]

For example, using the monoclonal antibody of this invention, 2, 3, 7, 8-TCDD can be separated from the mixture of other dioxin or similar organic compounds, and it can purify. If mixture is contacted to the monoclonal antibody of fixed this invention, the fixed complex of 2, 3, 7, 8-TCDD which the antibody combined is formed, after it dissociates from mixture and 2, 3, 7, 8-TCDD removes mixture, 2, 3, 7, 8-TCDD will be separated from an antibody, and a well-known technique will recover the thing of a high grade. or [fixing the constituent by this invention used in order to purify thru/or collect dioxin from complicated mixture on a permissible equipment] -- or it is made to mix with permissible support and the monoclonal antibody of this invention of the effective dose

of the condition in which a reaction and association with dioxin are possible is contained. The monoclonal antibodies of this invention are also the useful reagents for the research related to the structure and the operation function of dioxin. It becomes possible to use it for the immunochemical analysis of dioxin, and structure activity analysis for the sharp singularity which the antibodies of this invention have, and becomes what was suitable for singularity with the application like the above as compared with the polyclonal antibodies of a scarce conventional method. The constituent by this invention used as a research reagent contains the antibody of the amount which demonstrates the effectiveness of offering information by analysis following mixing with dioxin, and it. About the amount, it can decide suitably.

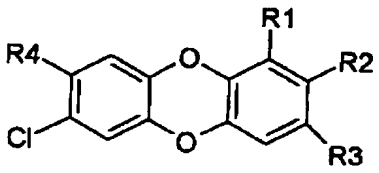
[0013]

It is as follows when production of the new monoclonal antibody of this invention and construction of the ELISA method are described in more detail.

1) Synthesis of a hapten antigen, a protein binding object, and an indicator object

About ten sorts of hapten antigens shown in Table 1 were synthesized in which a spacer having a different length and having a terminal carboxyl group through an acid amide, an ether, or a double bond is introduced at C-1 or C-2 of the dioxin frame.

[Table 1]



ハプテン	R1	R2	R3	R4
I－1	$NHCO(CH_2)_2COOH$	H	Cl	Cl
I－2	$NHCO(CH_2)_3COOH$	H	Cl	Cl
I－3	$NHCO(CH_2)_4COOH$	H	Cl	Cl
I－4	NHCOCH=CHCOOH	H	Cl	Cl
I－5	CH=CHCOOH	Cl	Cl	Cl
I－6	$CH=C(CH_3)COOH$	Cl	Cl	Cl
I－7	$NHCO(CH_2)_2COOH$	Cl	Cl	Cl
I－8	$NHCO(CH_2)_3COOH$	Cl	Cl	Cl
I－9	$NHCO(CH_2)_4COOH$	Cl	Cl	Cl
I－10	$(CH=CH)_2COOH$	Cl	Cl	Cl
II－1	H	OCH_2COOH	Cl	Cl
II－2	H	$O(CH_2)_3COOH$	Cl	Cl
II－3	H	$O(CH_2)_4COOH$	Cl	Cl
II－4	H	CH=CHCOOH	Cl	Cl
II－5	H	$(CH=CH)_2COOH$	Cl	Cl
II－6	H	CH=CHCOOH	H	Cl
II－6M	H	CH=CHCOOH	H	CH_3
II－6B	H	CH=CHCOOH	H	$C(CH_3)_3$

Chose several sorts of haptens from these, on the other hand, the connective which it is with bovine serum albumin (BSA) was made to react with a Western peroxidase (HRP) by the activity ester method again, and several sorts of enzyme-labeling objects were acquired.

[0014]

2) Monoclonal antibody

Repetitive immunity administration of the BSA connective of four sorts (I-2, I-3, I-5, II-2) of haptens was carried out at BALB/c or an A/J mouse, and the inhibition effectiveness according to TMDD about the individual which gave the result with the good anti-dioxin antibody titer in a blood serum investigated the affinity of an antibody further. Subsequently, the splenic cells obtained from two BALB/c mice with which the good result was obtained, and one A/J mouse, and a P3/NSI/1-Ag4-1 myeloma cell were united using polyethylene glucohol, and the culture supernatant of the syncytium obtained by HAT selective culture was screened by ELISA. Consequently, it was shown that the hybridoma around 200 sorts is secreting the anti-dioxin antibody.

3) Choose the hybridomas which produce an antibody having a greater inhibition effectiveness by TMDD and 2, 3, 7, 8-TCDD. Carry out cloning of the obtained hybridoma and a monoclonal antibody is prepared. As a result of examining many of the properties in a detail, it is a more specific monoclonal antibody than 1 or it of dioxin. It succeeded in obtaining the new monoclonal antibody whose compatibility is max among dioxin at 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin.

In the monoclonal antibody of this invention, some have a compatibility with (2) 1, 2, 3, 7, 8-pentachloro dibenzo-p-dioxin, (8) 2, 3, 7, 8-tetrachloro dibenzofuran and/or (10) 2, 3, 4, 7, 8-pentachloro dibenzofuran.

[0015]

[Examples]

I. The various conditions which are in charge of experimenting were as following.

1. Materials for Experiment

1) Hapten-Bovine-Serum-Albumin (BSA) Combination and Peroxidase (HRP) Indicator Hapten

Four-sort hapten (I-2, I-3, I-5, II-2) BSA combination, and eight-sort hapten (I-2, I-3, I-5, I-6, I-7, I-10, II-4, II-6) HRP indicator object as shown in Table 1 were used.

2) Mouse

BALB/c and A/J mice (all are a female and 8 week-old) were purchased from Japan SLC.

3) Myeloma cell strain

P3/NS1/1-Ag 4-1 Myeloma cell strain was provided by the Human Science Research Resource Bank.

[0016]

2. Reagent and Equipment

1) Immunity and ELISA Relation

Freund ** -- perfect and Freund's incomplete adjuvant: DIFCO 0638-60-7 and 0639-60-6 affinity purification rabbit anti-mouse IgG+IgM Antibody (second antibody): Jackson 315-005- 0441, 2, and 7-3 chlorination-8-methyl dioxin (TMDD) : Wellington Laboratorieso-phenylenediamine dihydrochloride: Sigma P902930% Hydrogen peroxide solution: Wako Pure Chem industry ELISA ** microtiter plate: Sumitomo Bakelite MS-9596F

2) Cell-fusion relation

RPMI 1640 powder culture medium: GIBCO-BRL 31800-022 fetal calf serum (FCS) : GIBCO-BRL 26140-079 hybridoma cloning factor (HCF) : IGENHAT Media Supplement: Sigma H0262 polyethylene glycol 4000 (PEG) : Merck Art 9727 dimethyl sulfoxide (DMSO): Sigma D2650 culture flask (25 cm2) : Iwaki Glass 3100-025 culture flask (75 cm2) : Becton Dickinson 3824 cluster dish (96 wells) : The special grade chemical was used for the salts, the organic solvent, etc. of Costar 3598 and others.

3. Device

ELISA plate reader (BL 312e) : Bio-Tek Instrument Inc.

4. immunity and the test blood collecting

Above-mentioned four-sort hapten (I-2, I-3, I-5, II-2) BSA It is each of combination by the following procedures BALB/c It carried out immunity administration each at a mouse and five A/J mice repeatedly. Hapten-BSA combination (50microg) Sterilization physiological saline (0.1 mL) It dissolves and is Freund's complete adjuvant. (0.1 mL) As an emulsion, it is foot pad (1 per one leg) of the above-mentioned mouse. And regions of back (hair clipper remove hair and they are about 20 places) It administered hypodermically. Henceforth, 6 - 8 times of boosters were performed for the immunogen of tales doses as an emulsion with Freund's incomplete adjuvant. A hematocrit tube is used [boosters / 5-7th] from ophthalmic veins seven days after, and it is test blood collecting. (20-40microL) It carried out. The blood serum was separated from the obtained blood with the conventional method, and reactivity with the HRP indicator dioxin was investigated by the following ELISA method.

[0017]

5. ELISA

1) Buffer solution and substrate solution

PBS:0.9% NaCl containing 0.05 mol/L NaH2PO4-Na2HPO4 Buffer solution (pH 7.3) Substrate solution: 0.05% o-Phenylenediamine-HCl and 0.01% The 50 mmol/L citric-acid-sodium acetate buffer solution containing a hydrogen peroxide (pH 5.0) .

2) Preparation of a second antibody fixed plate

96 hole ELISA microtiter plate -- each -- a well -- the second antibody PBS solution (2microg/mL) Distributive pouring (100microL/well) carrying out -- 4 degrees C -- overnight neglect. After carrying out suction removal of the antibody solution, a well is washed 3 times by PBS. BSA (0.5%) PBS (150microL/well) It pours distributively to a well and is left at a room temperature for 2 to 3 hours. PBS after carrying out suction removal of the solution The well was washed 3 times and the second antibody fixed plate was produced.

3) Approach of ELISA

Add the mouse blood serum, the hybridoma culture medium or mouse ascites, and enzyme-labeling dioxin which were diluted with PBS which contains 0.1% gelatin to the second antibody fixed plate (standard solution of dioxin is also added in inhibition trial) (100 microL/well), and it is overnight neglect at 4 degrees C. After carrying out suction removal of the solution, a well is washed 3 times by PBS and a substrate solution is added. (100microL/well) They are 30 minutes thru/or 1-hour neglect at a room temperature. 3 mol/L sulfuric acid (50microL/well) After suspending an enzyme reaction in addition, the absorbance of 490 nm was measured with the plate reader.

[0018]

6. Preparation of monoclonal antibodies by cell fusion

1) Media

Basal medium: 10 mmol/L HEPES-NaOH buffer solution (pH 7.3) and kanamycin sulfate (0.06%) are included. RPMI-1640.

Culture-medium: -- 10 % FCS and 0.05 mmol/L 2-mercaptoethanol and 2 mmol/L L-glutamine -- and -- 1 mmol/L Basal medium containing Bill Bin acid sodium.

HAT medium: 0.01 mmol/L Hypoxanthine and 16micromol/L Thymidine and 0.4micromol/L Culture culture medium containing aminopterin.

HT medium: 0.01 mmol/L Hypoxanthine and 16micromol/L Culture culture medium containing thymidine.

2) PEG solution

PEG (40 g) Dulbecco PBS (-) (50 mL) It dissolves and they are DMSO (10 mL) and 0.1%. Polly L-arginine hydrochloride solution (1 mL) After adding, pH was adjusted to about 7.5 using 1 mol/L NaOH.

3) Cell fusion

Physiological saline solution (0.5 mL) of corresponding immunogen (50microg) was intraperitoneally injected to the mouse with which the rise of antibody titer was accepted. A spleen is extracted the three days after and it is a basal medium. (10 mL) The splenic lymphocyte was unfolded in the put-in petri dish, and cell suspension was prepared. It is centrifugal at the room temperature after removing an explant using a stainless steel mesh. (1600 rpm, 6 minutes) It carries out and supernatant liquid is removed. It is a basal

medium to a pellet. (10 mL) A cell is made to suspend in addition, centrifugal is carried out on these conditions, and supernatant liquid is removed. It is a basal medium to the pellet after performing this actuation once [further]. (10 mL) In addition, the cell was suspended and the number of cells was calculated using that part. The above-mentioned splenic-cells suspension (whole quantity) It reaches, the myeloma cell of about 1/more than 5 is moved to a centrifuging tube, and it is centrifugal at a room temperature. (1600 rpm, 5 minutes) PEG solution beforehand warmed on the pellet at 37 degrees C after carrying out and fully removing supernatant liquid (1 mL) 1 minute was required and dropped. After mixing this cell suspension calmly for 1 minute, a basal medium is added in 3 steps. (1 mL 1 minute; 1 mL 1 minute; 8 mL 3 minutes) It is these conditions and is centrifugal. HAT medium which unfolds a pellet well after removing supernatant liquid, and contains HCF 10% (the used splenic cells 1 x per 108 pieces 50 mL) The cell after fusion actuation was made to suspend in addition. About this, it is distributive pouring (100 micro L/well) to a cluster dish 96 well. It carried out and 37 degrees C was cultivated by CO2 5%. The next day, HAT medium (100microL/well) It adds, suction removal of the abbreviation moiety of a culture medium will be carried out on the 6th for the 3rd day, and it is a HAT medium. (100microL/well) It newly added.

[0019]

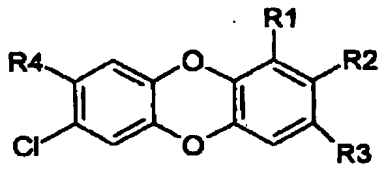
4) Screening of the anti-dioxin antibodies in a culture supernatant

Extract a part of culture supernatant of a hybridoma eight-ten days after the culture initiation, and it is BSA 0.1%. It contains. PBS It mixes and is above-mentioned second antibody immobilization. ELISA It added on the plate. After carrying out an incubation at a room temperature for 1 hour, suction removal of the solution was carried out and the plate was washed 3 times by PBS. HRP indicator dioxin (0.1microg; 100microL) After carrying out an incubation on these conditions in addition, the plate was washed similarly. each -- a well -- substrate solution (100microL/well) adding -- the above-mentioned approach -- plate top HRP Activity was measured. The hybridoma which had association of antibody-HRP indicator dioxin checked by TMDD (50 pg/well) was chosen from the hybridomas which are producing the antibody to dioxin. Furthermore, among them, hybridomas which are producing the antibodies of large inhibition effectiveness in (1) 2, 3, 7, 8-TCDD and (2) 1, 2, 3, 7, 8-PeCDD were screened, and hybridomas which produce monoclonal antibodies with strong compatibility to (1), D 2-37, D 9-36, and D 35-42 were obtained.

5) Cloning process

About each hybridoma chosen by screening, cloning was performed by limiting dilution. HT culture medium which contains HCF for a hybridoma 10% -- ten pieces / mL -- diluting -- the culture plate of 96 wells -- each -- a well -- every [0.2 mL] -- it poured distributively and cultivated for ten days. About the well as which growth of a hybridoma

is regarded, after checking that it is a single clone under a microscope, an antibody production hybridoma is transplanted to the culture plate of 48 wells, sequential growth was carried out, and single clone hybridomas, D 2-37, D 9-36, and D 35-42 were obtained.
[0020]
6) Description of a monoclonal antibody
The toxic equivalence multiplier and the rate of a crossover about three sorts of monoclonal antibodies which were obtained from three sorts of hybridomas obtained by the above 5 are shown in drawing 1 , and 2 and 3. The rate of a crossover **(ed) and calculated 2, 3, 7, 2, 3, 7 of each homolog computed from the calibration curve of 8-TCDD, and a 8-TCDD considerable amount with the addition of each homolog. As shown in drawing 1 -3, the three above-mentioned sorts of monoclonal antibodies have the maximum compatibility to (1) 2, 3, 7, 8-TCDD with the strongest toxicity in dioxins, and a high compatibility to (2) 1, 2, 3, 7, 8-PeCDD, (10) 2, 3, 4, 7, 8-PeCDF and/or (8) 2, 3, 7, 8-TCDF with a strong toxicity. The dioxin homolog content ratio in biological materials, such as mother's milk, has small individual difference, and since most of all TEQ(s) (toxic equivalent) are occupied by the homolog of said three sorts (1), (2), and (10), it is possible to carry out monitoring of the TEQ in a biological material by ELISA using this monoclonal antibody. Since especially the rate of a crossover with the dioxin homolog of D 9-36 is approximated with TEF (toxic equivalency factor), it has possibility that it is applicable also about the sample from which a homolog content ratio differs. Moreover, since D 35-42 recognizes only TCDD and PeCDD, a greatly different environmental sample of each homolog content may also be able to measure said two sorts of homologs. The rate of a crossover of a monoclonal antibody is as being shown in Table 2.
[0021]
[Table 2]



ハプテン	R1	R2	R3	R4
I－1	$NHCO(CH_2)_2COOH$	H	Cl	Cl
I－2	$NHCO(CH_2)_3COOH$	H	Cl	Cl
I－3	$NHCO(CH_2)_4COOH$	H	Cl	Cl
I－4	NHCOCH=CHCOOH	H	Cl	Cl
I－5	CH=CHCOOH	Cl	Cl	Cl
I－6	$CH=C(CH_3)COOH$	Cl	Cl	Cl
I－7	$NHCO(CH_2)_2COOH$	Cl	Cl	Cl
I－8	$NHCO(CH_2)_3COOH$	Cl	Cl	Cl
I－9	$NHCO(CH_2)_4COOH$	Cl	Cl	Cl
I－10	$(CH=CH)_2COOH$	Cl	Cl	Cl
II－1	H	OCH_2COOH	Cl	Cl
II－2	H	$O(CH_2)_3COOH$	Cl	Cl
II－3	H	$O(CH_2)_4COOH$	Cl	Cl
II－4	H	CH=CHCOOH	Cl	Cl
II－5	H	$(CH=CH)_2COOH$	Cl	Cl
II－6	H	CH=CHCOOH	H	Cl
II－6M	H	CH=CHCOOH	H	CH_3
II－6B	H	CH=CHCOOH	H	$C(CH_3)_3$

It is as follows when the description of three monoclonal antibodies is summarized.
D2-37: Since it has the highest compatibility to 2, 3, 7, 8-TCDD, and the rate of a crossover with other homologs is low, it can measure 2, 3, 7, 8-TCDD with the strongest toxicity in dioxins by specific and high sensitivity.
D9-36: Since it has a high compatibility to 1, 2, 3, 7, 8-PeCDD and 2, 3, 4, 7, 8-PeCDF besides 2, 3, 7, 8-TCDD, it can be used for monitoring of the dioxin toxicity equivalent in biological materials, such as mother's milk. Moreover, since the rate of a crossover with other homologs is also well correlated with TEF, it can be used also for toxic evaluation of an environmental sample etc.
D35-42: Since it reacts specifically with 2, 3, 7, 8-TCDD and 1, 2, 3, 7, 8-PeCDD, said two-sort dioxin homolog in all samples can be measured only by performing easy pretreatment.
[0022]
II. Standard curve of 2, 3, 7, 8-TCDD
Mixture of mouse ascites diluted with PBS containing 0.1% gelatin (D2-37: 200,000 times; D9-36:1,000,000 times; D35-42: 400,000 times) and I-5-HRP (0.4microg/mL) 90microL and 0.05% triton X-100 solution of 2, 3, 7, 8-TCDD (0. 4, 2, 10, 50, 250, 1250

pg/10microL) 10microL was added to each well of the second antibody fixed plate, and it was left at 4 degrees C overnight. It was hereafter operated like the above and the absorbance was measured. As shown in drawing 4, the measurement limitation of 2, 3, 7, 8-TCDD by the ELISA is around 1 pg, and improvement in the sensibility around 100 times was found as compared with the well-known approach.

[0023]

Comparison with well-known monoclonal antibodies

The rate of a crossover with dioxins and the isotype were compared with monoclonal antibodies to dioxins, DD-1, DD-3, DD-4, DD-5 and DD-6, described in the above-mentioned JP 63-14691A (USP No. 4,798,807) and shown in Table 3.

[Table 3]

		アイソタイプ		交差率(2,3,7,8-TCDD を 1 とした場合)						
ハイブリドーマ		クラス	L 鎖	(1)	(2)	(3)	(7)	(8)	(10)	(17)
引例	DD-1	IgG1	κ	1.00	6.25	0.10	< 0.01	2.50	0.40	< 0.01
	DD-3	IgG1	κ	1.00	3.13	0.13	< 0.01	3.57	8.33	< 0.01
	DD-4	IgG2a	κ	1.00	20.71	0.03	< 0.01	0.45	5.80	< 0.01
	DD-5	IgG2a	κ	1.00	3.00	< 0.09	< 0.05	10.00	6.00	< 0.05
	DD-6	IgG2a	κ	1.00	1.43	< 0.05	< 0.03	20.00	1.67	< 0.03
本発明	D2-37	IgG2a	κ	1.00	0.19	0.10	0.03	0.26	0.29	0.02
	D9-36	IgG1	κ	1.00	0.48	0.06	0.002	0.23	0.45	0.008
	D35-42	IgG2a	κ	1.00	0.96	< 0.005	0.000	< 0.005	< 0.005	0.0001

(1) : 2,3,7,8-TCDD
(2) : 2,3,4,7,8-PeCDD
(3) : 1,2,3,4,7,8-HxCDD
(7) : 1,2,3,4,5,6,7,8-OCDD
(8) : 2,3,7,8-TCDF
(10) : 2,3,4,7,8-PeCDF
(17) : 1,2,3,4,5,6,7,8-OCDF

As shown in Table 3,

(i) the antibodies of this invention differ in the rate of crossover with (2), (8) and (10), as compared with those describer in the known reference, and are new monoclonal antibodies.

(ii) Since the antibodies of this invention differ in the rate of crossover with analogous compounds, as compared with those describer in the known reference, correlation of measured value and the toxic equivalent differs in toxic evaluation.

(iii) Three sorts of monoclonal antibodies of this invention have the maximum compatibility to (1) 2, 3, 7, 8-TCDD with the strongest toxicity in dioxins, and their compatibility is high similarly to (2) 1, 2, 3, 7, 8-PeCDD, (10) 2, 3, 4, 7, 8-PeCDF and/or (8) 2, 3, 7, 8-TCDF with a strong toxicity. The dioxin homolog content ratio in biological materials, such as mother's milk, has small individual difference, and since most of all

TEQ(s) (toxic equivalent) are occupied by the homolog of said three sorts (1), (2), and (10), it is possible to carry out monitoring of the TEQ in a biological material by ELISA using this monoclonal antibody. Since especially the rate of a crossover with the dioxin homolog of D9-36 is approximated with TEF (toxic equivalency factor), it has possibility that it is applicable also about the sample from which a homolog content ratio differs. Moreover, since D35-42 recognizes only TCDD and PeCDD, a greatly different environmental sample of each homolog content may also be able to measure said two sorts of homologs. Although stated also in advance, three monoclonal antibodies of this invention have the following descriptions.

D2-37: Since it has the highest compatibility to 2, 3, 7, 8-TCDD, and the rate of a crossover with other homologs is low, it can measure 2, 3, 7, 8-TCDD with the strongest toxicity in dioxins by specific and high sensitivity.

D9-36: Since it has a high compatibility to 1, 2, 3, 7, 8-PeCDD and 2, 3, 4, 7, 8-PeCDF besides 2, 3, 7, 8-TCDD, it can be used for monitoring of the dioxin toxicity equivalent in biological materials, such as mother's milk. Moreover, since the rate of a crossover with other homologs is also well correlated with TEF, it can be used also for toxic evaluation of an environmental sample etc.

D35-42: Since it reacts specifically with 2, 3, 7, 8-TCDD and 1, 2, 3, 7, 8-PeCDD, said two-sort dioxin homolog in all samples can be measured only by performing easy pretreatment.

[0024]

[Effect of the Invention]

The monoclonal antibodies of this invention can be used for identification of the dioxin contained in the sample, and it can be used for the density measurement of the dioxin in a sample. As an object sample, biological materials, such as tissue of soil, water, fly ash, atmospheric air, and animals and plants, Homo sapiens blood, and mother's milk, etc. can be mentioned. the monoclonal antibodies of this invention, among dioxins, show almost equal compatibility to 2, 3, 7, 8-TCDD and 1, 2, 3, 7, 8-PeCDD with especially strong toxicity. They are so-called group-specific monoclonal antibodies and are useful in the high sensitivity primary simple screening procedure and the toxic monitoring method of dioxin contamination. Since the monoclonal antibody of this invention can supply the thing of fixed quality in large quantities and semipermanently, it is very useful also to development of the immuno affinity extraction method as a simple clean-up method of about establishment of immunoassay, and a test sample for chemical analysis. Moreover, in this invention, the ELISA method is established using the above-mentioned monoclonal antibody, since the measurement region of a standard solution is 1-1000pg, it can carry out the quantum of the dioxin of ultralow volume, and practicality is high [a region] to TEQ conversion exposure level evaluation of the dioxin in mother's milk

enough.

[0025]

[Brief Description of the Drawings]

[Drawing 1] It is the graph which shows the toxic equivalence multiplier and the rate of a crossover of the monoclonal antibody obtained from the hybridoma D2-37.

[Drawing 2] It is the graph which shows the toxic equivalence multiplier and the rate of a crossover of the monoclonal antibody obtained from the hybridoma D9-36.

[Drawing 3] It is the graph which shows the toxic equivalence multiplier and the rate of a crossover of the monoclonal antibody obtained from the hybridoma D35-42.

[Drawing 4] It is the graph which shows the sensitometry and the range of dioxin (2, 3, 7, 8-TCDD) by the ELISA method using the monoclonal antibody of this invention.

DRAWINGS

[Drawing 1]

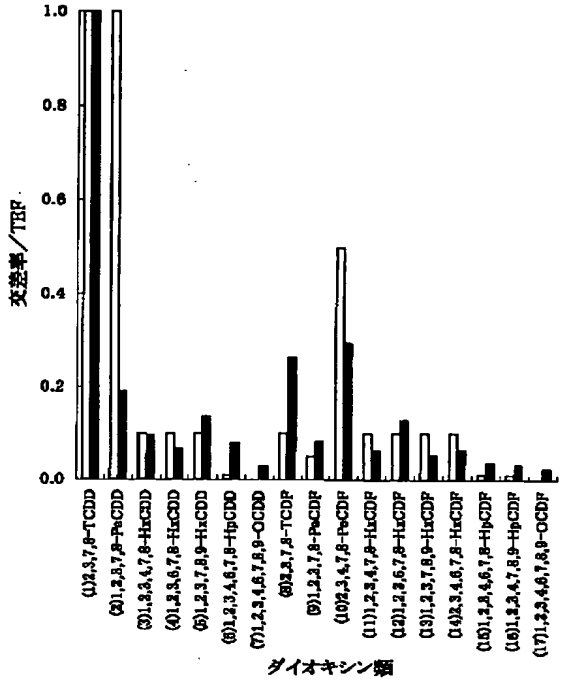


図1 D2－37の交差率(■)とTEF(□)

[Drawing 2]

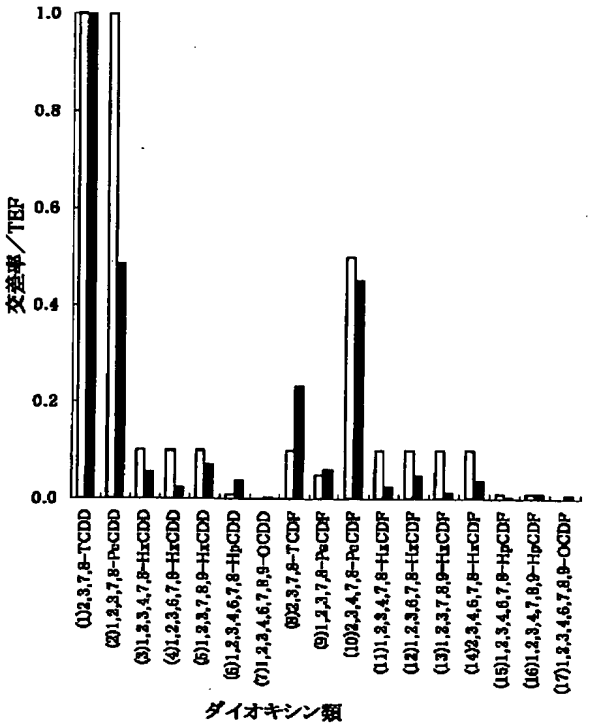


図2 D9-36の交差率(■)とTEF(□)

[Drawing 3]

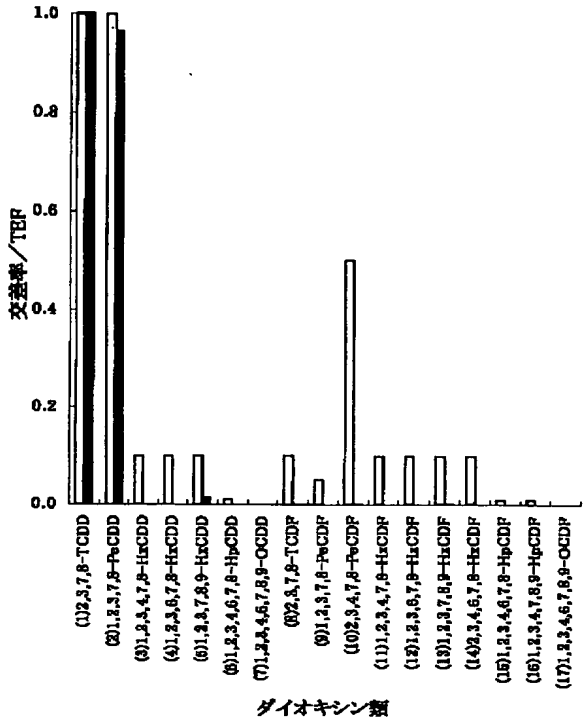
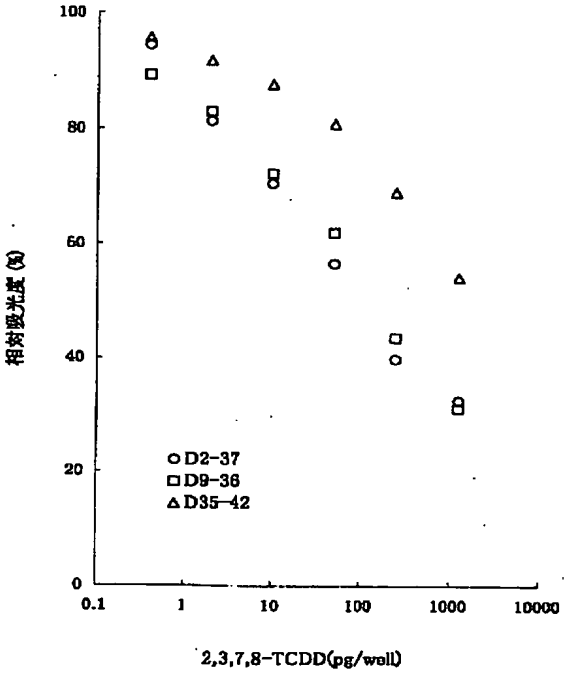


図3 D35－42の交差率(■)とTEF(□)

[Drawing 4]



（図4） 2,3,7,8-TCDD の標準曲線